REMARKS

Claims 1-65 are all the claims pending in the application. Claims 28-65 have been previously withdrawn.

Claim 1 has been amended to recite "wherein said antimicrobial agent is homogeneously distributed in the polymeric binder, such that the antimicrobial agent migrates through said polymeric binder." In view of the amendments to claim 1, claims 16 and 54 have been amended to delete the phrase, "and antimicrobial agents exhibiting the ability to migrate through said polymeric binder" and include the phrase "and mixtures thereof." Support for the amendment to claim 1 can be found in the specification at paragraphs [0042], [0046], and [0060]. Support for the amendment to claim 16 can be found in claim 54.

Claim 2 has been amended to delete the repeated term "marble" for clarification purposes as follows: "wherein said natural aggregate is selected from the group consisting of calcium carbonate, granite, quartz, feldspar, marble and quartzite and mixtures thereof," as requested by the Examiner.

Withdrawn claim 28 has been amended to depend from claim 1, and has additionally been amended for clarification purposes.

Thus, no new matter has been added herein. Entry of the Amendment is respectfully requested.

I. Response to Claim Objections

Claim 2 is objected to for allegedly containing informalities. Specifically, the Examiner requested correction of the phrase containing the word "marble" two times. Office Action at page 4.

As indicated above, claim 2 has been amended to delete one occurrence of "marble." Withdrawal of the objection to claim 2 is respectfully requested.

II. Response to Claim Rejections Under 35 U.S.C. § 102

Claims 1-13 and 19-27 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by WO/0023524 to Sakai et al. (U.S. Patent No. 6,750,283 is referenced for its English translation) ("Sakai"). Office Action at pages 4-5.

Applicants respectfully traverse for the following reasons.

The Examiner asserts that Sakai discloses a composite structural material comprising a natural aggregate (Sakai at col. 3, lines 45-50), a polymeric binder, a curing agent (Sakai at col. 7, lines 35-45), and an antimicrobial agent (Sakai at col. 8, lines 59-67). The Examiner contends that because the materials disclosed in Sakai allegedly are the same as instantly claimed, the composite of Sakai would have an appearance similar to that of natural stone. However, the Examiner admits (page 6 of the Action), Sakai fails to teach the use of an organic antimicrobial agent (OAA). Sakai merely discloses the use of an inorganic antimicrobial agent (IAA). See Sakai at col. 8, ll. 59-67. However, the inorganic antimicrobial agents used in Sakai are not soluble in resin, therefore, the inorganic antimicrobial agent will be disposed on the surface of the composite material as another inorganic aggregate. Claim 1 has been amended to recite that the antimicrobial is homogeneously distributed in the polymeric binder. Accordingly, the rejection is overcome and removal thereof is requested, respectfully.

III. Response to Claim Rejections Under 35 U.S.C. § 103

Claims 14-18 are rejected under § 103(a) as being unpatentable over Sakai, as applied to claims 1-13 and 19-27 above, in view of Schweizer et al., "Triclosan: a widely used biocide and its link to antibiotics" ("Schweizer") or EP 1428805 to Ramirez et al. ("Ramirez"). The

Examiner admits that Sakai fails to disclose an organic antimicrobial agent, and resorts to Schweizer and Ramirez as teaching the use of an organic antimicrobial agent in cementious composite mixtures. Office Action at pages 5-6.

Applicants respectfully traverse because the present invention provides a composite material having unexpectedly superior properties as compared to the products of any of Sakai, Schweizer or Ramirez, in that in the composite of the present invention the organic microbial agent is homogenously distributed in the polymeric binder. Thus, the antimicrobial agent can be present where resin is present.

In contrast, as discussed above, the inorganic antimicrobial agents used in Sakai are not soluble in resin. Therefore, the inorganic antimicrobial agent will be disposed on the surface of the composite material as another inorganic aggregate.

Similarly, both Schweizer and Ramirez disclose antimicrobial agents (including triclosan) that are insoluble in the water used to mix the cementatious material. More specifically, the organic antimicrobial agents of Schweizer and Ramirez have a very low polarity, and therefore are practically insoluble in polar media, such as water. In fact, triclosan is a hydrophobic agent. Therefore, the organic antimicrobial agents of Schweitzer and Ramirez would not homogeneously solubilize in the same manner as occurs in the present composition. Applicants submit U.S. Patent No. 5,955,408 column 1 from line 64, which discloses that triclosan has very poor solubility in water and therefore, requires organic solvents for solubilization. Applicants also submit the article *"Evaluation of a polymer coating containing triclosan as the antimicrobial layer for packaging materials"*, International Journal of Food Science & Technology. 38 (2):165-169, February 2003. Chung, Donghwan 1; Papadakis, Spyridon E. 2; Yam, Kit L. 3, which discloses the differences between triclosan solubility in organic solvents or water.

For an antimicrobial agent to function correctly, regardless whether it is inorganic or organic, it is not enough to merely adjust its concentration. In fact, the manner in which the antimicrobial agent reacts and its layout or arrangement in the material is crucial for its functionality. The present inventors found that for the antimicrobial agent to function optimally, it must be on the surface where it functions and distributed uniformly in the resin so that more can migrate to the surface as the antimicrobial agent on the surface is depleted. Thus, for the presently claimed composite material, in order to decide what dose to add to ensure that the desired results are obtained, Applicants had to take into account whether the resin would be exposed on the surface and whether the antimicrobial agent was soluble in the resin. This is not appreciated by or disclosed in any of Sakai, Schweizer and Ramirez. It is also necessary to know if migration is possible, and if so, how fast the agent can migrate to the surface of the composite. This also is not disclosed in or appreciated by any of Sakai, Schweizer and Ramirez.

This is an important difference between the cited references and the present invention. As the specification indicates in paragraphs [0042] and [0046], the Applicants are actually looking for the antimicrobial agent's migration through the polymeric net and as the description discloses, there can be differences in the migration speed between different polymers depending on the crosslink degree. Moreover, paragraphs [0054] and [0060] disclose that there is a need for the antimicrobial agent mixture to be uniformly mixed into the overlap composition. The specification even discloses the option of preparing a pre-mixture of the antimicrobial agent with the binder in order to facilitate the homogenization.

Finally, different studies show that when triclosan is consumed and decomposed in a cementious aqueous environment, eventually triclosan would lose its antimicrobial properties. Specifically, the presence of chlorophenols and other waste compounds in aqueous solutions

(especially in a basic medium) suggest the decomposition of triclosan. Applicants provide *"Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products."* Environmental Toxicology and Chemistry. Article: pp. 517-525 for support. Thus, the presence of chlorophenols and other waste compounds will make the concentration optimization difficult in the products of Schweizer and Ramirez. For this reason, also, the present invention, which uses an organic antimicrobial agent and a resin binder has unexpectedly superior properties as compared to the products of Schweizer and Ramirez.

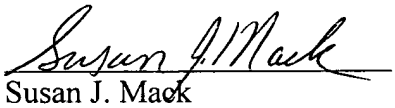
In view of the above, the Examiner is requested, respectfully, to reconsider and remove the rejection over Sakai, in view of Schweizer or Ramirez, be withdrawn.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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United States Patent [19]

Kaiser et al.

[11] Patent Number: 5,955,408

[45] Date of Patent: Sep. 21, 1999

[54] **TRICLOSAN SKIN WASH WITH ENHANCED EFFICACY**

[75] Inventors: **Nancy E. Kaiser**, Pontoon Beach, Ill.; **Denise K. Pretzer**, Chesterfield, Mo.

[73] Assignee: **STERIS Inc.**, Temecula, Calif.

[21] Appl. No.: **08/890,521**

[22] Filed: **Jul. 9, 1997**

Related U.S. Application Data

[60] Provisional application No. 60/021,453, Jul. 10, 1996.

[51] **Int. Cl.**[6] **C11D 1/12**; C11D 3/44; C11D 3/48

[52] **U.S. Cl.** **510/131**; 510/119; 510/123; 510/125; 510/130; 510/235; 510/237; 510/342; 510/382; 510/386; 510/405; 510/407; 510/414; 510/432; 510/434; 510/471; 510/477; 510/480

[58] **Field of Search** 510/119, 123, 510/125, 130, 131, 235, 237, 342, 382, 386, 405, 407, 414, 432, 434, 471, 477, 480

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,975,218	12/1990	Rosser	252/117
5,006,529	4/1991	Resch	514/721
5,057,311	10/1991	Kamegai et al.	424/70
5,364,031	11/1994	Taniguchi et al.	239/330
5,415,810	5/1995	Lee et al.	252/545
5,417,875	5/1995	Nozaki	252/106
5,635,469	6/1997	Fowler et al.	510/406
5,681,802	10/1997	Fujiwara et al.	510/130
5,716,626	2/1998	Sakurai et al.	424/401

FOREIGN PATENT DOCUMENTS

2202698	10/1973	France .
3723990 A1	7/1987	Germany .
1408885	10/1975	United Kingdom .
WO 96/06153	of 0000	WIPO .
WO 93/07250	4/1993	WIPO .
WO 95/09605	4/1995	WIPO .
WO 95/24179	9/1995	WIPO .
WO 95/32705	12/1995	WIPO .
WO 96/06152	2/1996	WIPO .

Primary Examiner—Yogendra Gupta
Assistant Examiner—Charles Boyer
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[57] **ABSTRACT**

A disinfectant hand wash includes triclosan and a reduced amount of surfactants in order to reduce skin irritation while maintaining improved activity against Gram negative and Gram positive organisms, including *Serratia marcescens*. The hand wash includes an effective amount of triclosan, preferrably 0.2–3.0% and a non-aqueous solvent. A mixture of hexylene glycol and isopropanol provide improved efficacy in killing skin-born microbes. The hand wash further includes 2–20% surfactant, preferrably at less than 10%. The hand wash further includes a chelating agent, a thickener, a buffering agent, and water.

14 Claims, No Drawings

Evaluation of a polymer coating containing triclosan as the antimicrobial layer for packaging materials

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Summary A coating made of a styrene-acrylate copolymer and containing triclosan (87 ± 9 mg triclosan cm^{-3} coating) was evaluated as an antimicrobial layer for packaging materials. In both agar diffusion tests and liquid culture tests, inhibition of growth of *Enterococcus faecalis* by the triclosan-coating was observed. Triclosan in the coating was not released into the water. Using a solution of 10% aqueous ethanol to simulate aqueous/acidic foods, a very small portion of the triclosan was quickly released with an estimated apparent partition coefficient of 1.7×10^{-5}. When using *n*-heptane, to simulate fatty foods, most of the triclosan was extracted with an estimated apparent partition coefficient of 5.5×10^{-2}.

Keywords Bacterial inhibition, *Enterococcus faecalis*, release kinetics, styrene-acrylate copolymer.

Introduction

During the past decade, there has been great interest in developing antimicrobial packaging materials to prevent microbial food contamination of food surfaces during storage, by slowly releasing antimicrobial agents such as organic acids, potassium sorbate, imazalil, benzoic anhydride and hexamethylenetetramine (Ghosh *et al.*, 1977; Vojdani & Torres, 1990; Rico-Pena & Torres, 1991; Weng & Hotchkiss, 1992, 1993; Chen *et al.*, 1996; Han & Floros, 1998; Devlieghere *et al.*, 2000; Ouattara *et al.*, 2000).

Recently, we developed a fungistatic packaging material by coating a styrene-acrylate copolymer emulsion containing propyl paraben onto a packaging paper (Chung *et al.*, 2001a,2001b). The coating was effective in inhibiting *Saccharomyces cerevisiae*, and the observed release of propyl paraben was controlled by Fickian diffusion within the coating. In this study, we substituted propyl paraben with another antimicrobial agent, triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether).

Triclosan is an outstanding, tasteless, odourless and orally non-toxic bacteriostatic agent (Bhargava & Leonard, 1996). It is currently used as an active ingredient in Colgate® toothpaste (Colgate-Palmolive Co., NY) and Microban® plastic kitchen utensils (Microban Intl. Ltd, NY), including chopping boards. Its minimum inhibitory concentration against bacteria is very low; for example, it ranges from 3 to 10 mg L^{-1} against *Enterococcus faecalis* on brain–heart infusion agar (Bhargava & Leonard, 1996). For these reasons, triclosan may be a good candidate for developing antimicrobial packaging materials.

The objective of this study was to evaluate a styrene-acrylate copolymer coating containing triclosan as the antimicrobial layer for packaging materials. The bacterial inhibition by the coating, and the release kinetics of triclosan from the coating into water and food simulating solvents were examined. *Enterococcus faecalis* was used as an indicator, because it can cause nosocomial infections and spoilage in meats, cheese, surface waters and vegetables (Franz *et al.*, 1999).

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Materials and methods

Preparation of polymer coating containing triclosan

A styrene-acrylate copolymer coating containing about 10% (w/w) triclosan was prepared using the method described by Chung *et al.* (2001a,2001b). One gram of triclosan (2,4,4′-trichloro-2′-hydroxy diphenyl ether, Princeton Polymer, Union, NJ, USA) was added into 20.9 g of a water-based styrene-acrylate emulsion (Carboset®GA-1604; B.F. Goodrich Specialty Chemicals Co., Cleveland, OH, USA). The mixture was agitated with gentle heating for 2 h, and then it was manually coated on a clay-coated packaging paper (13 × 15 cm; International Paper Co., Tuxedo, NY, USA) using a special rod (R.D. Specialties, Inc., Webster, NY, USA). The triclosan-coated paper was then dried at 94–96 °C for about 1 min. A triclosan-free coating was also prepared as the control. The average thickness of coating was 46 ± 8 μm, measured using a digital micrometer (Digitrix II, Japan Micrometer MFG Co., Osaka, Japan). To determine the amount of triclosan in the coating, three coated papers were cut into 6.25 cm diameter discs. Each disc was immersed in 100 mL ethanol (Pharmaco Products, Brookfield, CT, USA) overnight with shaking, and the amount of extracted triclosan was determined using HPLC. The concentration of triclosan in the coating was 87 ± 9 mg cm^{-3}.

Determination of triclosan concentration

The HPLC system used for triclosan detection consisted of a pump (model 590; Waters Associates, Milford, MA, USA), a reverse phase C-18 column (Superisorb ODS2, 10 cm × 4.6 mm i.d., 3 μm particle size; Supelco Inc., Bellefonte, PA, USA), and a UV detector (model 2050; Varian Associates, Sunnyvale, CA, USA). A 60 : 40 (v/v) acetonitrile/water mixture was pumped at 1 mL min^{-1}, and triclosan was detected at $\lambda = 254$ nm with 8-min retention time.

Bacterial culture and media

Enterococcus faecalis (ATCC 19433) was maintained in tryptic soya broth (TSB) (Difco Laboratories, Detroit, MI, USA) at 30 °C by daily transfer. Tryptic soya agar (TSA) (Difco Laboratories) was used as a solid medium.

Bacterial inhibition test

Bacterial inhibition by the coated papers was evaluated using an agar diffusion test and a liquid culture test. The agar diffusion test made use of coated papers cut into 5 cm diameter discs. A volume of 5 mL of melted TSA was poured into a Petri dish. Then, just before the agar solidified, a sample disc was placed onto the agar with the coated side up, this prevented the paper from curling. *Enterococcus faecalis* was cultured overnight in 5 mL of TSB at 30 °C with agitation, and the culture was diluted with melted TSA at 42–45 °C to yield a cell concentration of *c.* 10^2 CFU mL^{-1}. A volume of 8 mL of the melted TSA containing *E. faecalis* cells was then poured onto the agar plate to form the second layer. Duplicate agar plates were prepared for both triclosan coated paper and control paper. The agar plates were incubated at 30 °C for 48 h.

For the liquid culture test, the triclosan coated papers were cut into 2 × 2.5 cm rectangles. Five sample rectangles (25 cm^2 total surface area) were immersed in 20 mL TSB in a 50-mL flask. The medium was inoculated with 200 μL of *E. faecalis* culture in its late exponential phase, and then transferred to a shaking incubator at 30 °C and 200 r.p.m. The culture was sampled (500 μL size) periodically during the incubation to obtain microbial growth profiles. The same procedure was used for testing the control paper. To determine the concentration of *E. faecalis* cells, each culture sample was appropriately diluted with distilled water and its optical density ($o.d._{600}$) was measured at $\lambda = 600$ nm using a spectrophotometer (Hitachi Ltd, Tokyo, Japan).

Release test

The release kinetics of triclosan from the coating was examined using the diffusion cell described by Chung *et al.* (2001b). A volume of 100 mL of water or solvents simulating particular food was poured into the cell and agitated. The temperature

of the solvents was maintained at 30 °C by circulating hot water through the outside jacket of the cell. After conditioning the coated paper, a 6.9-cm diameter disc was cut and placed at the bottom of the cell. The sample disc surface area exposed to the solvents was 30.68 cm^2 (6.25 cm diameter). For simulating aqueous/acidic foods and fatty foods, a 10% (v/v) aqueous ethanol mixture and *n*-heptane (Fisher Scientific Co., Pittsburgh, PA, USA) were used, respectively (Chung *et al.*, 2001b). Time interval sampling (1 mL size) was conducted, and the triclosan concentration in each sample was determined using the HPLC.

Results and discussion

Inhibition of *E. faecalis*

In the agar diffusion test, inhibition of *E. faecalis* was clearly shown. Colonies of *E. faecalis* were not observed in the circular region directly above the triclosan coated paper, while colonies were formed all over the control plates. The microbial inhibition indicates that a small portion of triclosan was released from the coating, diffused into the agar layer, and then inhibited the microbial cells embedded within the agar layer. Although triclosan is not soluble in pure water, it may be slightly soluble in the water held by the agar because of the presence of some hydrophobic substances.

In liquid culture, the inhibition of *E. faecalis* by the coating with triclosan was also clearly shown (Fig. 1). At the stationary growth phase, the cell concentration in the control medium ($o.d._{600}$ = 3.5) was 1.5 times higher than the cell concentration in the medium containing the coating with triclosan ($o.d._{600}$ = 1.4), although the lag times (about 3 h) were almost the same. For comparison purposes, the specific cell growth rates during the exponential growth phase were also calculated using:

$$dX(t)dt = \mu X(t) \quad (1)$$

where $X(t)$ is the cell concentration of *E. faecalis* in the medium ($o.d._{600}$), μ is the specific growth rate of *E. faecalis* (h^{-1}), and t is time (h). The μ value of the cells affected by triclosan (0.36 h^{-1}) was only 60% of the value for the control (0.60 h^{-1}).

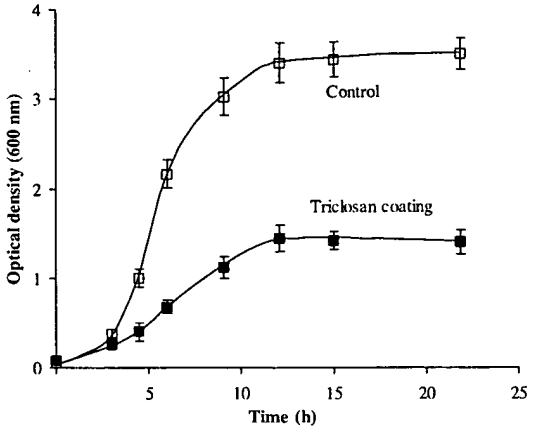

Figure 1 Inhibition of *Enterococcus faecalis* by the polymer coating containing triclosan in a liquid culture medium at 30 °C (vertical bars represent standard deviations of three replicates).

Release kinetics of triclosan

Using pure water, no release of triclosan was observed (0.1 mg L^{-1} detection limit), because of its insolubility. In 10% aqueous ethanol, about 1.2% of triclosan was quickly released from the coating, and equilibrium was observed within 20 h (Fig. 2). This quick release could be caused by the interactions between the coating and the aqueous ethanol (Baner *et al.*, 1994). The calculated apparent partition coefficient (defined as the ratio of triclosan concentration in the solvent to that in the coating at equilibrium) is *c.* 1.7×10^{-5}. This value is much less than unity, indicating that triclosan is much more attracted to the coating than to the solvent. The results suggest that the amount of triclosan released from the coating to aqueous/acidic foods is small. However, this small amount may be still sufficient for microbial inhibition as suggested by the results of microbial inhibition test and the reported low minimum inhibitory concentrations of triclosan.

Using *n*-heptane, about 65% of the triclosan was quickly released from the coating, which suggests the coating was strongly affected by *n*-heptane. The remaining triclosan was slowly released until about 97% was extracted. The large amount of release was because of the high solubility of triclosan in *n*-heptane (triclosan is readily soluble in organic solvents). The calculated

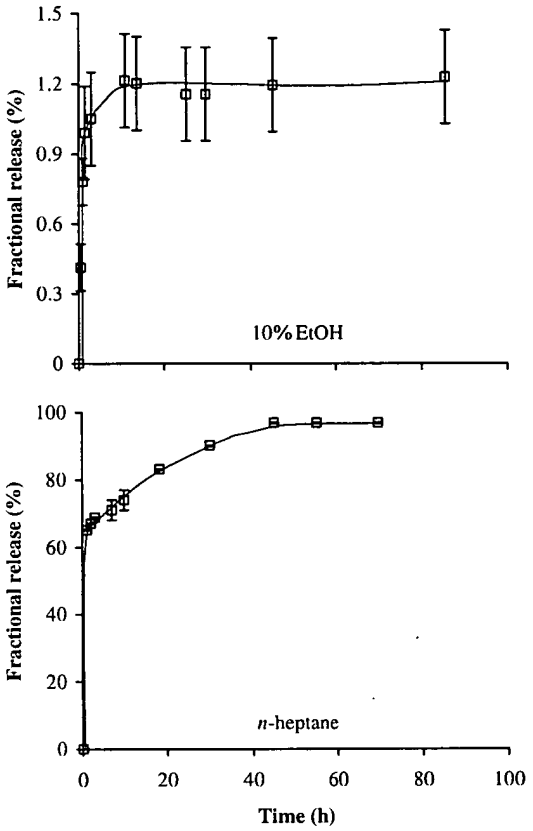

Figure 2 Release of triclosan from the polymer coating into 10% aqueous ethanol and *n*-heptane at 30 °C (vertical bars represent standard deviations of three replicates).

apparent partition coefficient is *c.* 5.5×10^{-2}, which is much larger than the value for 10% aqueous ethanol. However, this value is still smaller than unity, indicating higher triclosan affinity to the coating. This result suggests that the coating may not be suitable for fatty food applications because of the quick release of a large amount of triclosan. Note that the experiments were at 30 °C, and the rate of release will be smaller at refrigerated temperatures.

Conclusion

The data suggested that a styrene-acrylate copolymer containing triclosan could be an effective antimicrobial layer, under appropriate conditions. The bacterial inhibition by the coating was clearly demonstrated by an agar diffusion test and a liquid culture test. The release kinetics of triclosan depended on the solvent and varied greatly, from almost no release (for water) to quick release (for *n*-heptane). Future research is needed to evaluate the effectiveness of packaging materials containing triclosan against other micro-organisms.

References

Baner, A.L., Franz, R. & Piringer, O. (1994). Alternative fatty food simulants for polymer migration testing. In: *Food Packaging and Preservation* (edited by M. Mathlouthi). Pp. 23–47. New York: Blackie Academic & Professional.

Bhargava, H.N. & Leonard, P.A. (1996). Triclosan: applications and safety. *American Journal of Infection Control*, **24**, 209–218.

Chen, M., Yeh, G.H. & Chiang, B. (1996). Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *Journal of Food Processing and Preservation*, **20**, 379–390.

Chung, D., Chikindas, M.L. & Yam, K.L. (2001a). Inhibition of *Saccharomyces cerevisiae* by slow release of propyl paraben from a polymer coating. *Journal of Food Protection*, **64**, 1420–1424.

Chung, D., Papadakis, S.E. & Yam, K.L. (2001b). Release of propyl paraben from a polymer coating into water and food simulating solvents for antimicrobial packaging applications. *Journal of Food Processing and Preservation*, **25**, 71–87.

Devlieghere, F., Vermeiren, L., Jacobs, M. & Debevere, J. (2000). The effectiveness of hexamethylenetetramine-incorporated plastic for the active packaging of foods. *Packaging Technology and Science*, **13**, 117–121.

Franz, C.M.A.P., Holzapfel, W.H. & Stiles, M.E. (1999). Enterococci at the crossroads of food safety? *International Journal of Food Microbiology*, **47**, 1–24.

Ghosh, K.G., Srivatsa, A.N., Nirmala, N. & Sharma, T.R. (1977). Development and application of fungistatic wrappers in food preservation. Part II. Wrappers made by coating process. *Journal of Food Science and Technology*, **14**, 261–264.

Han, J.H. & Floros, J.D. (1998). Simulating diffusion model and determining diffusivity of potassium sorbate through plastics to develop antimicrobial packaging films. *Journal of Food Processing and Preservation*, **22**, 107–122.

Ouattara, B., Simard, R.E., Piette, G. *et al.* (2000). Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science*, **65**, 768–773.

Rico-Pena, D.C. & Torres, J.A. (1991). Sorbic acid and potassium sorbate permeability of an edible methylcellulose-palmitic acid film: water activity and pH effect. *Journal of Food Science*, **56**, 497–499.

Vojdani, F. & Torres, J.A. (1990). Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: effect of fatty acids. *Journal of Food Science*, **55**, 841–846.

Weng, Y. & Hotchkiss, J.H. (1992). Inhibition of surface molds on cheese by polyethylene film containing the antimycotic imazalil. *Journal of Food Protection*, **55**, 367–369.

Weng, Y. & Hotchkiss, J.H. (1993). Anhydrides as antimycotic agents added to polyethylene films for food packaging. *Packaging Technology and Science*, **6**, 123–128.

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AQUEOUS PHOTOCHEMISTRY OF TRICLOSAN: FORMATION OF 2,4-DICHLOROPHENOL, 2,8-DICHLORODIBENZO-*p*-DIOXIN, AND OLIGOMERIZATION PRODUCTS

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Abstract—The photochemical fate of the antimicrobial agent triclosan is presented. Experiments performed in both natural and buffered deionized water show that triclosan rapidly photodegrades by direct photolysis ($t_{1/2}$ = 5 h, pH 8, noon summer sunlight, 45°N latitude). Both 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) and 2,4-dichlorophenol (2,4-DCP) are produced. The 2,8-DCDD and 2,4-DCP also are photolabile and, thus, are intermediates. The yields for 2,8-DCDD and 2,4-DCP ranged from 3 to 12% depending on the conditions employed. When triclosan is photolyzed in the presence of Suwannee River (GA, USA) fulvic acid, a portion of the initial mass is recovered as insoluble material. Based on experiments in which the formation of insoluble material was monitored with photolysis time, it is postulated that photolysis in natural waters leads to some of the triclosan being coupled to humic matter. Triclosan also reacts rapidly with both singlet oxygen (k_{rxn} = 1.07 ± 0.03 × 10^8 M^{-1} s^{-1} in water of pH 10) and hydroxyl radical ($k_{\cdot OH}$ = 5.4 ± 0.3 × 10^9 M^{-1} s^{-1}). Indirect photolysis pathways, however, are not expected to be important because of low steady-state concentrations of reactive oxygen species in natural waters and the efficiency of the direct photolysis of triclosan.

Keywords—Triclosan Dioxin Dichlorophenol Photochemistry Reactive oxygen species

INTRODUCTION

The environmental fate of organic wastewater compounds, including pharmaceuticals and personal care products (PPCPs), is receiving increased scrutiny following the detection of numerous PPCPs in surface waters throughout Europe, South America, and the United States [1–8]. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a widely employed antibacterial compound that is used in a variety of consumer products. In a recent reconnaissance for a suite of PPCPs, the U.S. Geological Survey detected triclosan in 57% of the 139 streams tested [1]. This is particularly troublesome for a number of reasons. First, a mechanism for bacterial resistance to triclosan recently has been elucidated [9]. Second, triclosan is structurally similar to other polychlorinated phenoxyphenols that have been shown to cyclize to the toxic polychlorodibenzo-*p*-dioxins [10–15]. These findings have led researchers to study the fate of triclosan in the aquatic environment [2,6,8,16–19].

Recent studies have shown that photolysis pathways are responsible for the transformation of certain pharmaceuticals in natural waters [2,5–8,16,20,21]. In the case of triclosan (pK_a = 7.9 [22]), direct phototransformation is responsible for 80% of the epilimnetic loss of triclosan in Lake Greifensee during summer [6,8]. Aqueous photolysis of triclosan, albeit with high-energy ultraviolet (UV) light (254 nm), leads to hydrolysis, dechlorination, and catechol products [19]. Aqueous solutions of triclosan can be converted to 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) by high-energy UV light (254 nm) [15]. In a preliminary account of the present work, we showed that triclosan is photochemically converted to 2,8-DCDD in natural water with environmentally relevant wavelengths of light (λ > 300 nm) [16].

To gain a more complete understanding of the environmental photolysis pathways, it is important to identify the products of the photoreactions and to determine the relevance of indirect photolysis mechanisms. In the present study, we add 2,4-dichlorophenol (2,4-DCP) to the suite of triclosan photolysis products. To our knowledge, no studies have been reported concerning the reaction of triclosan with the environmentally important reactive oxygen species singlet oxygen ($^1O_2$), which is highly reactive toward a variety of phenolic substrates [23–25], and hydroxyl radical (·OH), a nonselective transient species that reacts at near diffusion-controlled rates with most organic compounds [26,27]. The present study demonstrates that triclosan reacts readily with both $^1O_2$ and ·OH. In addition, the present study extends our previous examination of the formation of 2,8-DCDD, including two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopic characterization of the dioxin product and determination of the formation quantum yield under natural sunlight. Finally, we provide evidence that one sink for triclosan in natural waters may be photocoupling to humic material.

MATERIALS AND METHODS

Chemicals

Triclosan, furfuryl alcohol (FFA), acetophenone, *p*-nitroacetophenone, butylated hydroxytoluene (BHT), and Rose Bengal (RB) were purchased from Aldrich (Milwaukee, WI, USA). Diethanolamine, 2,4-DCP, isoprene, sodium azide, *m*-methoxyacetophenone, and D_2SO_4 were purchased from Acros Organics (Pittsburgh, PA, USA). Both D_2O and NaOD were purchased from Cambridge Isotope Labs (Andover, MA, USA).

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Sodium molybdate dihydrate was purchased from Baker (Phillipsburg, NJ, USA). A 2,8-DCDD standard was purchased from NeoSyn Laboratories (New Milford, CT, USA). Polystyrene sulfonate standards were purchased from Polysciences (Warrington, PA, USA). Suwaneee River fulvic acid (SRFA) was acquired from the International Humic Substances Society (St. Paul, MN, USA). Isoprene was purified by vacuum distillation to separate it from radical inhibitors; all other chemicals and solvents were used as received. Solvents were of high-performance liquid chromatography (HPLC) grade.

Natural water analysis

Mississippi River water (MRW) was collected at Hastings (MN, USA; collected spring 2002; pH 8.0; dissolved organic carbon, 8.0 mg/L; see Latch et al. [20] for cation and anion concentrations), and Lake Josephine water (LJW) was collected at Roseville (MN, USA; collected autumn 2002; pH 8.4; dissolved organic carbon, 5.8 mg/L; see [28] for cation and anion concentrations). Both were analyzed for total organic carbon by a Tekmar Dohrmann, Phoenix 8000 instrument (Mason, OH, USA). Filtered subsamples (pore size, 0.45 μm) were analyzed for anions using a Dionex (Sunnyvale, CA, USA) ion chromatography system with an AS14 column and carbonate-buffered eluent. Cations were determined with a ThermoElemental PQ inductively coupled plasma–mass spectrometer (Waltham, MA, USA). The water was filtered (pore size, 0.2 μm) before use in photolysis experiments.

Photolysis procedures

In laboratory experiments using artificial light at ambient temperatures (~30°C), two separate designs were used. Experiments quantifying triclosan (3–100 μM) or 2,8-DCDD (0.73–1.3 μM) decay kinetics and 2,8-DCDD growth were performed on a merry-go-round apparatus in which aqueous samples (25 or 50 ml, buffered to pH 4–12 or in MRW) were placed in quartz bottles and irradiated with a 450-W, medium-pressure, Hg-vapor lamp (Ace Glass, Vineland, NJ, USA) controlled by an Ace Glass 7830 power supply. The lamp was placed inside of borosilicate or quartz cooling wells (Ace Glass). Alternatively, a turntable apparatus was used in which samples (triclosan or 2,4-DCP, 100 μM) were irradiated in buffered deionized (DI) water, LJW, or MRW with four 175-W, medium-pressure, Hg-vapor lamps (LumaPro lamps, Grainger, Minneapolis, MN, USA; GE HR175A39/CP bulbs; General Electric, CT, USA) as the light source. Sample solutions were placed in quartz tubes (outer diameter, 1.3 cm; inner diameter, 1.1 cm; V = 10 ml) oriented at approximately 30° to the light source. Experiments in natural sunlight were performed in a similar fashion, with 100 μM triclosan samples in pH 8–buffered DI water or natural water in 10-ml quartz tubes. The tubes were capped with cork stoppers and placed at an angle of approximately 30° to the incident sunlight. These experiments were performed in late summer and early autumn in Minneapolis (MN, USA; 45°N latitude). In kinetic analyses, small aliquots of sample were withdrawn at predetermined intervals of time and analyzed by HPLC.

A pyridine/*p*-nitroacetophenone actinometer [29] solution was photolyzed alongside the sample solutions to determine the intensity of light reaching the samples. Quantum yields were calculated using the spectral overlap integral (UV–visible spectra were collected with a Jasco V-530 spectrophotometer [Easton, MD, USA]), after correcting for screening of triclosan and triclosan anion at the maximum of the action spectrum (λ = 313 nm).

The involvement of indirect photochemical processes were assessed in the laboratory using artificial light by photolyzing 100 μM triclosan in LJW (pH 8) or in DI water spiked with SRFA (1–16 mg/L) side-by-side with a triclosan solution in DI water. The DI water and SRFA-spiked samples were buffered (borate) to pH 8 to match the pH of the natural water sample. Triplet sensitizer and quencher experiments also were performed, in which acetone (10% v/v), *m*-methoxyacetophenone (360 μM), or isoprene (1.7 mM) were spiked into the samples or the samples were deoxygenated before photolysis. The free radical–quencher butylated hydroxytoluene (0.3 molar equivalents) was added to triclosan samples to test for the involvement of radical species in the conversion to 2,8-DCDD. Fluorescence spectra were recorded (Jasco FP6200 instrument) to analyze for radiative deactivation processes of the triclosan excited state.

In analyzing polymer formation and the coupling of triclosan to humic matter, large sample volumes (0.5–1 L) were irradiated for approximately 16 h under four 175-W, Hg-vapor lamps. Samples of triclosan (10 or 100 μM) alone or of triclosan (10 μM) in the presence of SRFA (10.2 mg/L) dissolved in DI water adjusted to pH 10 were irradiated and then analyzed for the formation of insoluble photocoupled products by filtering the photolysates through a nylon membrane (pore size, 0.2 μm; Millipore®, Billerica, MA, USA). Control studies also were performed in which unphotolyzed samples or photolyzed SRFA (10.4 mg/L) in the absence of triclosan were processed in the same manner.

Singlet oxygen reaction

Laser-flash photolysis. The home-built instrument used in the present study has been described previously [20]. Triclosan samples were analyzed in diethanolamine-buffered D_2O (pD 9–12; pD ~ $-\log[D^+]$).

Steady-state photolysis. The turntable apparatus described above was used to irradiate triclosan samples containing various concentrations of substrate (20–50 μM) and 40 μM RB, a well-defined $^1O_2$ sensitizer, dissolved in pH 10 carbonate or pH 5 acetate buffers. These experiments were conducted in borosilicate tubes, which screen some of the UV (<300 nm) intensity and minimize direct photolysis of the triclosan (the triclosan phenolate anion has an absorbance maximum at 295 nm and tails off at wavelengths > 300 nm) but allow the RB sensitizer (λ_{max} = 559 nm) to absorb the longer-wavelength light. Samples of FFA (50–200 μM, buffered at pH 5 or 10), a $^1O_2$-probe molecule with known reactivity, along with 40 μM RB were photolyzed concurrently with the triclosan samples. Aliquots of sample were withdrawn at various intervals and analyzed by HPLC. Quenching studies in which triclosan (20 μM) was photolyzed in the presence of various concentrations of sodium azide (0.33–2.6 mM), a selective $^1O_2$ scavenger, were performed as a control to ensure that $^1O_2$ was the active species causing the triclosan decay.

Reaction of triclosan with thermally generated $^1O_2$. The reaction of triclosan with $^1O_2$ was monitored using ^1H NMR spectroscopy (NMR spectra were recorded with either Varian Inova 300-, 500-, or 600-MHz instruments in D_2O solvent; Varian, Palo Alto, CA, USA). The high substrate concentrations necessary for NMR spectroscopic detection prevented the use of photochemical means for producing $^1O_2$. Instead, $^1O_2$ was produced from the molybdate-catalyzed disproportia-

tion of D_2O_2 [30]. In a NMR tube, D_2O_2 (300 μmol) was added to a basic D_2O solution of triclosan (40 μmol) and Na_2MoO_4 (90 μmol), with EtOH (36 μmol) as an internal standard. After 20 min, a NMR spectrum was obtained.

Preparation of 5-chloro-2-(2,4-dichlorophenoxy)quinone. The *p*-quinone analogue of triclosan was prepared following the general procedure for the oxidation of phenols using $PhI(O_2CCH_3)_2$ described by Saitz et al. [31]. The ^1H NMR spectroscopy ($CDCl_3$) δ 7.54 (d; J = 2.4), 7.36 (dd; J_1 = 3.0, J_2 = 8.7), 7.13 (d; J = 8.7), 7.06 (s), and 5.75 (s). The ^{13}C NMR spectroscopy ($CDCl_3$) δ 179.1, 178.7, 157.2, 147.0, 145.4, 133.5, 131.9, 131.4,129.2, 128.3, 123.8, and 110.0. Ultraviolet–visible spectra λ_{max} = 269 nm, and $\varepsilon = 2.7 \times 10^4$ M^{-1} cm^{-1}. Additionally, IR $\nu_{C=O}$ = 1,680 cm^{-1}. High resolution mass spectrometry m/z = 302.9375 ($M + H^+$).

Hydroxyl radical reaction

The second-order rate constant for the reaction of triclosan with hydroxyl radical was determined using Fenton's reagent as described previously [20].

Chromatographic conditions

High-performance liquid chromatography. Chromatograms were obtained with an 1100 Series Hewlett-Packard (Avondale, PA, USA) HPLC with a UV-absorbance detector controlled by Chemstation software (Foster City, CA, USA). A ThermoFinnigan liquid chromatography quadrupole-ion storage Advantage HPLC-MS with Xcalibur software (Herndon, VA, USA) also was used for mass spectral analysis of the aqueous photolysate. Triclosan, 2,8-DCDD, 2,4-DCP, and the quinone analogue of triclosan were analyzed on a Supelco Discovery RP-Amide C_{16} column (Supelco, Bellefonte, PA, USA) using an 80:20 acetonitrile (ACN):pH 5 acetate–buffered mobile phase run at a flow rate of 1 ml/min. The detector wavelength was set at 280 nm for the first 6 min of each run and to 230 nm thereafter. To minimize the effect of a small, interfering peak, additional analyses of 2,4-DCP growth and decay used a 50:50 ACN:pH 5 acetate buffer as the mobile phase. Furfuryl alcohol samples were analyzed on a Phenomenex ODS-2 column (Phenomenex USA, Torrance, CA, USA) using a 70:30 ratio of the ACN:pH 5 acetate buffer. The flow rate was set at 1 ml/min, and the detector wavelength was set to 219 nm.

Size-exclusion chromatography. Molecular weight (MW) determinations were based on size-exclusion chromatography (SEC) as described by Chin et al. [32]. Size-exclusion chromatograms were obtained using a Waters Protein Pak 125 column (Waters, Milford, MA, USA) and a 0.1 M NaCl, 5 mM phosphate, pH 7 buffer. The flow rate was 1 ml/min, and the detector was set to a wavelength of 230 nm. Polystyrene sulfonate standards and an acetone sample were used to calibrate the retention times. Triclosan photolysis samples were analyzed in the same manner as the standards, and the MW range of the photolysis products were estimated based on the calibration curve of the standards.

Gas chromatography–mass spectrometry. An Agilent 6890 series gas chromatography (GC) system (Agilent, Palo Alto, CA, USA) equipped with a 5973 mass-selective detector was used to obtain total-ion chromatograms and mass spectra on injecting the samples onto a Hewlett-Packard 5MS column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm). The oven was held at 40°C for 4 min, then ramped to 250°C at 20°C/min and held for an additional 10 min. The mass spectrometer (MS) was operated in both the scan and selective-ion mode (m/z = 252, 189, and 126 for 2,8-DCDD) and used to detect triclosan, 2,8-DCDD, 2,4-DCP, and 2,4-dichloroanisole in the aqueous photolysate.

RESULTS AND DISCUSSION

Direct photolysis

Triclosan decay kinetics. When photolyzed under Hg-vapor lamps (λ > 300 nm), the triclosan phenolate anion rapidly degraded, whereas the parent phenol form displayed much slower photodegradation kinetics ($k_{pH\ 11}/k_{pH\ 4}$ = 20). These results are consistent with those in other reports of triclosan photoreactivity [2,6,8,16]. This behavior can, in part, be rationalized by the UV absorbance spectra of the two species: The parent phenol has a peak maximum at 280 nm and very little absorbance above 300 nm, whereas the phenolate form has a peak maximum centered at 295 nm and a significant tail at wavelengths greater than 300 nm. The phenolate form absorbs much more light than the parent phenol, because sunlight and the lamps used in the laboratory photolysis experiments (when borosilicate filtered) exhibit very little emission at less than 300 nm, leading to faster degradation rate constants for the anionic form. A rapid communication of this work concerning the kinetics of triclosan decay reported quantum yields calculated under conditions of varying triclosan concentration, pH, and irradiation wavelength [16]. Quantum yields were calculated using the spectral overlap integral (i.e., using the discrete emission lines from the Hg-vapor lamps or, for the solar spectrum, at the wavelength intervals outlined by Leifer [29]) by comparison to the pyridine/*p*-nitroacetophenone actinometer [29]. The quantum yields were corrected for screening of triclosan and triclosan anion according to the method described by Zepp and Cline [33] using the absorbance at the action spectra maxima (313 nm). Table 1 summarizes results from the present study, data from the aforementioned rapid communication [16], and a study by Tixier et al. [8], in which the pH and wavelength dependence of triclosan photodegradation was assessed.

In sunlight, triclosan (pH 8, DI water) photodegraded with a half-life of 2.5 h in midsummer (Φ_{-T} = 0.12) (Table 1). After correcting for the lens effect [34,35], the half-life was 5 h. The quantum yield measured with sunlight irradiation is lower than that measured under Hg-vapor lamps, reflecting differences in the light sources. The high direct photochemical quantum yields in alkaline solutions and the short half-life measured indicate that photochemistry may be an important loss process for triclosan in the environment. This idea is in agreement with the results of previous field and modeling studies [6,8], in which direct photochemical loss processes alone were the major summertime sinks for triclosan in the epilimnion of Lake Greifensee.

The triplet sensitizers acetone and *m*-methoxyacetophenone did not cause an increase in triclosan degradation rate. In fact, triclosan photodegradation was slightly slower with these species present because of light screening. Removal of oxygen, a potent quencher of excited-state triplet species, did not alter the rate of triclosan loss. Likewise, addition of the triplet-quencher isoprene or the radical-quencher BHT did not affect the triclosan decay rate. These results suggest that the triclosan degradation pathway does not involve a radical chain mechanism or an excited triplet state. The most likely reactive triclosan species therefore is the excited singlet state. In an attempt to quantify any other potential deactivation routes of the

Table 1. Quantum yields for triclosan degradation (Φ_{-T}) and 2,8-dichlorodibenzo-*p*-dioxin (DCDD) formation (Φ_{DCDD}), 2,8-DCDD degradation (Φ_{-DCDD}), 2,4-dichlorophenol (DCP) formation (Φ_{DCP}), and 2,4-DCP degradation (Φ_{-DCP}) and yields of 2,8-DCDD and 2,4-DCP formation in direct photolysis experiments

			% 2,8-DCDD			
[Triclosan] (μM)	pH	Irradiation conditions	Φ_{-T}	Φ_{DCDD}	Yield	Source
2	11.5	λ = 313 nm	0.50	ND[a]	ND	[8]
2	11.0	λ = 292 nm	0.31	ND	ND	[8]
2	5.9	λ = 279 nm	0.40	ND	ND	[8]
21	11.5	Hg-vapor, Pyrex filter	0.32	3.9×10^{-2}	12	[16]
18.4	8.0	Hg-vapor, Pyrex filter	0.74	3.0×10^{-2}	4.1	[16]
3.4	8.0	Hg-vapor, Pyrex filter	0.73	3.0×10^{-2}	4.1	[16]
100	8.0	Sunlight	0.12	ND	ND	Present study
			% 2,8-DCDD			
[2,8-DCDD] (μM)	pH	Irradiation conditions	Φ_{-DCDD}			Source
0.73	8.0	Hg-vapor, <280 nm filter	5.9×10^{-3}			[16]
1.3	8.0	Hg-vapor, <320 nm filter	1.4×10^{-3}			[16]
			% 2,4-DCP			
[Triclosan] (μM)	pH	Irradiation conditions	Φ_{-T}	Φ_{DCP}	Yield	Source
100	8.2	Hg-vapor, Pyrex filter	0.73	2.3×10^{-2}	3.1	Present study
			% 2,4-DCP			
[2,4-DCP] (μM)	pH	Irradiation conditions	Φ_{-DCP}			Source
100	8.2	Hg-vapor, Pyrex filter	0.65			Present study

[a] ND = not determined.

excited-state triclosan, fluorescence experiments were conducted. It was found that neither the parent nor the phenolate form of triclosan was fluorescent, indicating that the remainder of the deactivation of triclosan results from nonradiative pathways.

Intermediacy of 2,8-DCDD and 2,4-DCP. Analysis of the soluble fraction of the triclosan photolysate has led to the characterization of two potentially harmful products, 2,8-DCDD and 2,4-DCP. Chlorinated dioxins, as a class, have drawn considerable interest among environmental researchers because of their toxicity [10,36–39]. Likewise, the formation of 2,4-DCP is a concern, because it is a U.S. Environmental Protection Agency priority pollutant. The kinetics of the formation and subsequent photodegradation of these species therefore were analyzed more closely. In both cases, the photoproducts were photolabile.

*2,8-Dichlorodibenzo-*p*-dioxin.* In the analysis of the direct photolysis products by HPLC, a well-separated peak eluting after the triclosan parent compound was identified as 2,8-DCDD by comparison with an authentic standard. A previous communication regarding this work reported the formation of 2,8-DCDD in laboratory photolysis studies [16]. The present study also confirms (by HPLC retention time comparison to an authentic sample of 2,8-DCDD) that 2,8-DCDD is formed when triclosan is photolyzed under sunlight ($\Phi_{-T} = 0.12$). The most compelling evidence for 2,8-DCDD formation is provided by a 2D $^1H/^{13}C$ heteronuclear multiple–quantum coherence NMR spectrum in CD_3OD (Fig. 1) of the hexanes extract of the aqueous triclosan photolysate. The spectrum indicates that triclosan and 2,8-DCDD are the primary components of the extract.

The quantum yield for 2,8-DCDD formation (Φ_{DCDD}) and the 2,8-DCDD product yield (%; Φ_1/Φ_{-T}) from triclosan, as determined from the kinetic data in Latch et al. [16], are summarized in Table 1. Results of experiments in which 2,8-DCDD was independently photolyzed illustrate that it readily photodegrades. It is evident from the quantum yields and kinetic profiles summarized in Table 1 and from the data of Latch et al. [16] that 2,8-DCDD is an intermediate in the photolysis of triclosan (Fig. 2). In experiments performed in the laboratory, 2,8-DCDD appears and then degrades as the photolysis proceeds.

2,4-Dichlorophenol. In addition to the hydrophobic 2,8-DCDD peak in the HPLC chromatograms, a series of small, earlier eluting peaks appeared following triclosan photolysis. One of these peaks corresponded to 2,4-DCP (based on the retention time of an authentic sample of 2,4-DCP). To characterize further the presence of 2,4-DCP in triclosan photolysis solutions, the photolysate was analyzed by GC-MS following acidification, extraction into ethyl acetate, and derivatization with diazomethane, a common methylating agent [40]. The derivative 2,4-dichloroanisole (m/z = 177) of 2,4-DCP was detected in the extract.

When monitoring triclosan photolysis solutions for 2,4-DCP, it was apparent that 2,4-DCP was a reactive intermediate, because it displayed growth and decay kinetics (Fig. 3). The analysis of 2,4-DCP growth kinetics differed from that used to detect 2,8-DCDD growth because of the similar photodegradation kinetics of 2,4-DCP and triclosan. The relatively fast photodegradation of the 2,4-DCP produced in the triclosan photolysis experiments precluded measuring the 2,4-DCP growth kinetics in the same manner as that for 2,8-DCDD, because the formation and degradation of 2,4-DCP occur on the same time scale. Therefore, in the kinetic analysis of 2,4-DCP emerging from the triclosan photolysis, the data were fit

to the following equations (Scientist for Windows, Ver. 2.01; Micromath Scientific Software, Salt Lake City, UT, USA):

$$\frac{d[\text{triclosan}]}{dt} = -(k_{DCP} + k_{other})[\text{triclosan}] \quad (1)$$

$$\frac{d[\text{DCP}]}{dt} = k_{DCP}[\text{triclosan}] - k_{-DCP}[\text{2,4-DCP}] \quad (2)$$

where k_{DCP} is the rate constant for the formation of 2,4-DCP from triclosan and k_{-DCP} is the rate constant for the subsequent loss of 2,4-DCP. The degradation rate constant for 2,4-DCP was determined by photolyzing an authentic sample under the same conditions and observing decay (pH 8, $k_{-DCP} = 5.9 \times 10^{-3}$ M^{-1} s^{-1}). This value was used in Equation 2 to solve for k_{DCP} (pH 8, 1.3×10^{-5} M^{-1} s^{-1}). These data were used to calculate the quantum yield of 2,4-DCP formation ($\Phi_{DCP} = 2.3 \times 10^{-2}$) (Table 1), degradation ($\Phi_{-DCP} = 0.65$), and product yield (%; Φ_{DCP}/Φ_{-T}) in the same manner as that described above.

The low yield for 2,4-DCP formation in the photolysis of triclosan (3.1%), taken together with the two orders of magnitude faster k_{-DCP} than k_{DCP}, suggests that the 2,4-DCP formed from this process will lead only to low steady-state concentrations (<250 pM based on the product yield of 2,4-DCP formation and the detection of triclosan at concentrations up to 8 nM in natural waters [1]) in the environment. The photolability of 2,4-DCP observed in this work reinforces the work of Boule et al. [36,41], who also have shown 2,4-DCP to be photolabile. In addition to illustrating the photochemical decay of 2,4-DCP in aqueous solutions, those authors determined that it primarily forms a mixture of three isomeric chlorocyclopentadiene carboxylic acids and their dimerization products [41]. These ring contraction compounds are expected to be the products of 2,4-DCP photolysis in both the triclosan and 2,4-DCP experiments in the present work (Fig. 2). Retention time analysis of the earliest eluting peaks of the triclosan and 2,4-DCP photolysis solutions indicate that the same products likely are present in the two photolysates.

Formation of polymers and coupling to dissolved organic matter. Despite performing many extractions and chromatographic and spectroscopic analyses on the triclosan photolysate, we were unable to identify any other triclosan photodegradation products, and the triclosan mass balance was low. We hypothesize that triclosan may form photocoupled products (with other triclosan molecules and dissolved organic matter) that may account for some of the triclosan mass balance. During irradiation, the triclosan samples underwent photoyellowing, as evidenced by UV–visible spectra absorbance growth at 330 nm. The rate constant for the absorbance growth correlates well with the triclosan decay rate and indicates a humification process. Size-exclusion chromatography was used to test for the formation of coupled products. Following photolysis of 10 μM triclosan in alkaline DI water and concentrating the photolysate 10-fold by removing the water in vacuo, samples were analyzed by SEC. Unphotolyzed triclosan (MW = 289) did not yield any significant peaks in its SEC chromatogram, but the photolysate chromatogram showed a distribution of broad, overlapping peaks, which are indicative of the formation of higher-MW species. By comparison of the photolysate SEC chromatogram with those of the polystyrene sulfonate standards, the MW range of the products was calculated to be approximately 100 to 1,700 g/mol, indicating that the coupling products are relatively small oligomers and

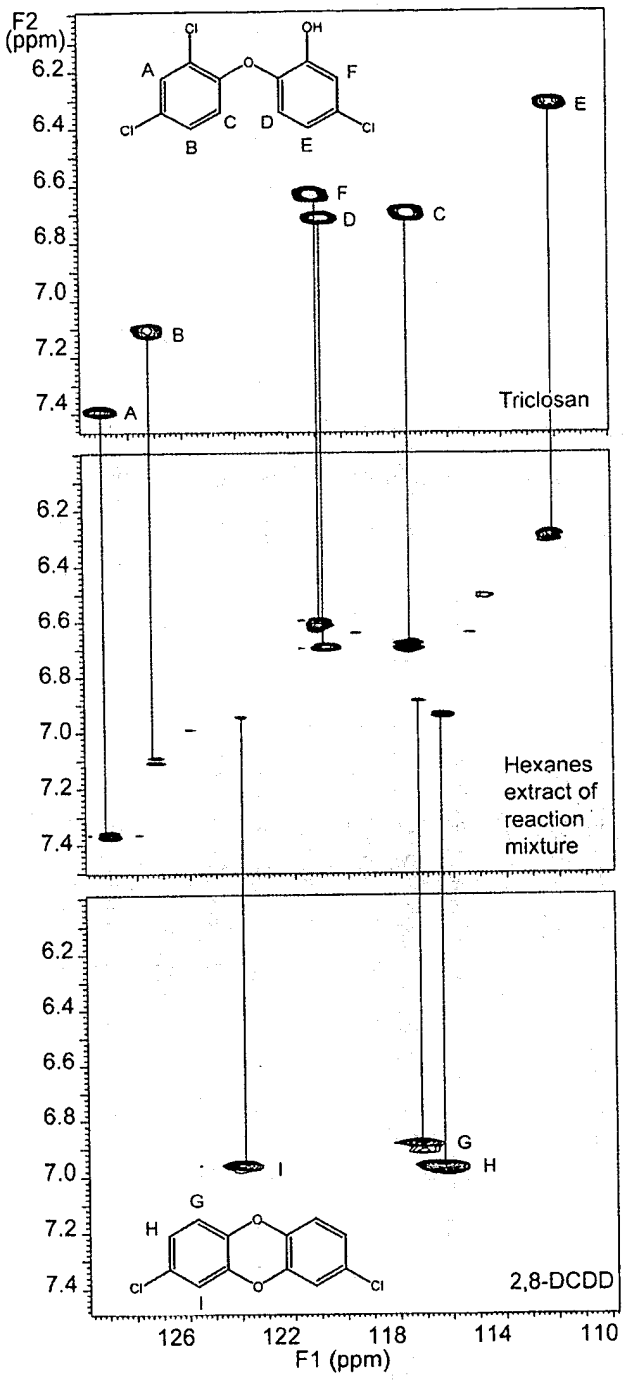

Fig. 1. Downfield region of $^1H/^{13}C\{^1H\}$ heteronuclear multiple-quantum coherence nuclear magnetic resonance spectra (500 MHz) of triclosan (top; $CD_3OD/NaOD$), 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD; bottom; CD_3OD), and a mixture resulting from the photolysis of triclosan in water (middle; $CD_3OD/NaOD$). The middle spectrum is that of a sample obtained by hexane extraction of the aqueous photolysate of triclosan. The F1 dimension is ^{13}C chemical shift (ppm), and the F2 dimension is ^1H chemical shift (ppm).

polymers. A comparison between chromatograms of the photolysate and an 1,800-MW polystyrene sulfonate standard is shown in Figure 4. Examples of triclosan-derived oxidative coupling products are provided in the work of Zhang and Huang [42]. The photocoupled species formed from triclosan

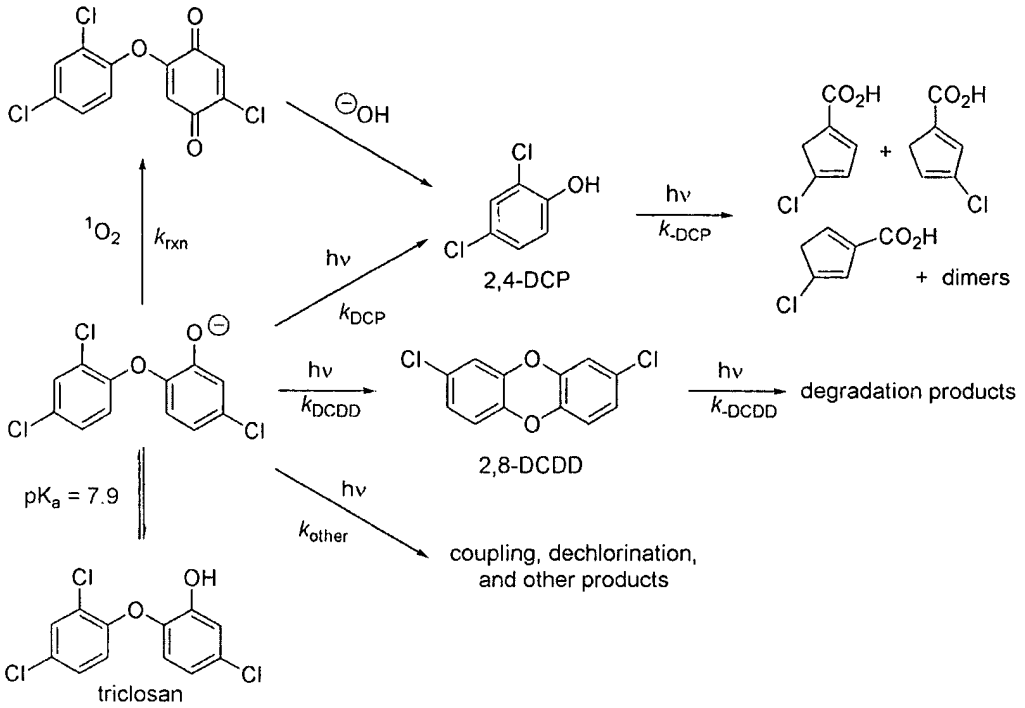

Fig. 2. Photochemical reaction pathways of triclosan in water based on the present study and [10,16,19,24,41]. DCDD = dichlorodibenzo-*p*-dioxin; DCP = dichlorophenol.

likely are similar to the dimeric species that Zhang and Huang proposed for the manganese oxide–mediated oxidation of triclosan. Triclosan does appear to undergo condensation reactions at relatively high concentrations, but it is unlikely that it would condense with itself in natural waters given the low concentrations found in the environment (typically <8 nM [1]) and the rate of the self-coupling reaction is expected to decrease approximately with the square of the triclosan concentration.

When triclosan was photolyzed alone at a relatively high concentration (100 μM), a sizeable portion (19%) of the initial starting mass was trapped on a filter membrane (pore size, 0.2 μm) following irradiation. At lower concentrations (10 μM), a negligible amount (≤0.1 mg of the initial 5.7 mg of triclosan) of insoluble products from the photolysate was trapped on the filter membrane when triclosan was photolyzed alone. To test whether coupling to dissolved organic matter occurs, triclosan (10 μM) also was photolyzed in the presence of SRFA (10.2

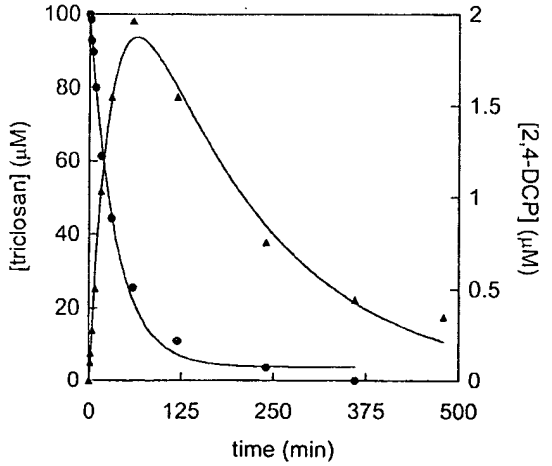

Fig. 3. Triclosan decay (circles) and 2,4-dichlorophenol (2,4-DCP) growth and decay (triangles) in the irradiation of triclosan (pH 8.2) under four 175-W, Hg-vapor lamps (Pyrex filtered). Note the separate concentration scales. Triclosan decay was fit to Equation 1, and the 2,4-DCP growth and decay kinetics were fit to Equation 2.

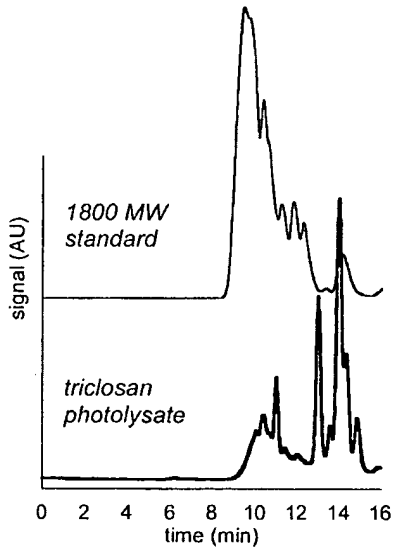

Fig. 4. Size-exclusion chromatogram of triclosan (10 μM) photolyzed under Hg-vapor lamps and concentrated 10-fold in vacuo (lower trace). A chromatogram of an 1,800-molecular weight (MW) polystyrene sulfonate standard, used to calibrate the MW/retention time relationship, is shown for comparison (upper trace). Signal intensity is measured in absorbance units (AUs).

Table 2. Reactivity of triclosan (pK_a = 7.9) with singlet oxygen ($^1O_2$) and hydroxyl radical (•OH) in H_2O or D_2O

	$^1O_2$		•OH	
pH	k_{rxn} (10^8 M^{-1} s^{-1}) measured in H_2O by SSP[a]	k_{tot} (10^8 M^{-1} s^{-1}) measured in D_2O by LFP[b]	pH	$k_{•OH}$ (10^8 M^{-1} s^{-1}) in H_2O by Fenton's reaction
5	0.03 ± 0.004[c]	NM[d]	3.5	54 ± 3
9	NM	1.3 ± 0.2		
10	1.07 ± 0.06	2.1 ± 0.2		
11	NM	2.1 ± 0.2		
12	NM	2.2 ± 0.2		

[a] SSP = steady-state photolysis.
[b] LFP = laser-flash photolysis.
[c] Error range represents the 95% confidence interval.
[d] NM = not measured.

mg/L). No measurable amount of insoluble particles were trapped when the SRFA was photolyzed in the absence of triclosan, but a small amount of insoluble material (accounting for 7% of the total mass of triclosan and SRFA in the initial solution) was trapped on the membrane when triclosan was present in the SRFA photolysis solution. These insoluble particles are postulated to arise from photoinduced coupling of triclosan to the SRFA and are further indication that humification occurs.

Indirect photolysis

Singlet oxygen. Laser-flash photolysis and steady-state photolysis experiments were both used to assess the kinetics of triclosan and $^1O_2$ interaction. These complementary techniques provide information about how the substrate is interacting with $^1O_2$.

Laser-flash photolysis. Laser-flash photolysis was used to measure the general reactivity (i.e., the sum of chemical reaction and physical quenching; $k_{tot} = k_{rxn} + k_{phys}$) of triclosan with $^1O_2$. Experimental details and the kinetic model have been described previously [20]. The samples were analyzed in D_2O because of the much longer $^1O_2$ lifetime (60 vs 4 μs) than in H_2O [25]. Triclosan samples were analyzed over the pD range of 9 to 12, yielding k_{tot} values ranging from 1.35 to 2.2 × 10^8 M^{-1} s^{-1} (Table 2). It is apparent that the triclosan phenolate anion interacts rapidly with $^1O_2$ over this pD range. It should be noted that the low solubility of triclosan in more acidic media prevented the measurement of k_{tot} at lower pD values, because laser-flash photolysis requires relatively high substrate concentrations.

Steady-state photolysis. Steady-state photolysis experiments probed the specific reactivity (i.e., chemical reaction) of triclosan toward $^1O_2$. In these experiments, loss of substrate peak area is monitored by HPLC. The kinetic model in which a steady-state approximation for $^1O_2$ is made has been presented previously [20]. The reaction rate constant (k_{rxn}) is determined by comparing the observed rate constant for triclosan disappearance ($k_{obs,triclosan}$) to that for FFA ($k_{obs,FFA}$), which is a singlet oxygen probe with a known reaction rate constant (8.3 × 10^7 M^{-1} s^{-1}) [20], under the same conditions (Eqn. 3):

$$k_{rxn,triclosan} = \frac{k_{obs,triclosan} k_{rxn,FFA}}{k_{obs,FFA}} \quad (3)$$

This analysis yields k_{rxn} = 1.07 ± 0.03 × 10^8 M^{-1} s^{-1} at pH 10 (>99% in the phenolate anion form) and 3.0 ± 0.01 × 10^6 M^{-1} s^{-1} at pH 5 (>99% in the parent phenolic form; $k_{pH\ 10}/k_{pH\ 5}$ = 36), as seen in Table 2. At intermediate pH values, k_{rxn} can be calculated based on the speciation of triclosan (pK_a = 7.9) and these end-member rate constants. The effect of pH on k_{rxn} can be rationalized by the fact that $^1O_2$ has been shown to be much more reactive toward electron-rich phenolate anions than to undissociated phenolic compounds [23]. Addition of azide, which is a known $^1O_2$ quencher, caused a predictable suppression in the rate of triclosan disappearance.

Singlet oxygen reaction product analysis. During triclosan reactions in which $^1O_2$ was photochemically generated, 2,4-DCP was observed as a product by both HPLC and GC-MS. Analysis of the reaction between triclosan and thermally generated $^1O_2$ (MoO_4^{2-} + D_2O_2) by NMR spectroscopy also demonstrated that 2,4-DCP was the main product. This method could not be used to determine a yield for this reaction, because the 2,4-DCP itself reacts with $^1O_2$ [23]. The conversion of triclosan to 2,4-DCP by $^1O_2$ therefore was determined by HPLC analysis of triclosan decay and 2,4-DCP growth during the initial stage of RB-sensitized photolysis experiments in which a filter solution ($NaNO_3$; wavelength, <330 nm) was used to minimize direct photolysis of triclosan. The yield was calculated by dividing the number of moles of 2,4-DCP formed by the moles of triclosan lost and was found to be 79%.

Most reports of phenol singlet oxygenation reactions indicate that *p*-quinones are the resultant products [23,24]. No quinone product was observed in the analysis of the $^1O_2$ and triclosan reaction mixtures by GC-MS, HPLC, and NMR spectroscopy. When subjected to the aqueous environments in which the $^1O_2$ reactions were performed, however, the independently synthesized 5-chloro-2-(2,4-dichlorophenoxy)quinone standard spontaneously decomposed to 2,4-DCP. We therefore postulate that the quinone is a short-lived intermediate in the $^1O_2$ reaction (Fig. 2).

Hydroxyl radical. Using the methods described previously [20], the reaction between triclosan and hydroxyl radical was studied via competition with a reference compound with a known hydroxyl radical rate constant (acetophenone, $k_{•OH}$ = 5.9 × 10^9 M^{-1} s^{-1} [26]). A bimolecular rate constant of 5.4 ± 0.3 × 10^9 M^{-1} s^{-1} for triclosan at pH 3.5 was calculated. This rate constant, determined at the optimum pH for the Fenton reaction, is not expected to be very sensitive to pH, because it already is near the diffusion-controlled limit. Hydroxyl radical is a nonspecific transient that could react with triclosan in a variety of ways, including H-atom abstraction and substitution.

Kinetics of triclosan photodegradation in natural water or with SRFA sensitizer. When photolyses were conducted in SRFA-spiked DI water samples, the triclosan decay rates matched those from the photolyses performed in DI water alone. The rate in LJW (pH 8) was lower (k_{LJW}/k_{DI} = 0.6) than that in DI water (pH 8.6), reflecting the pH dependence of triclosan direct photodegradation in alkaline solutions and the light screening caused by the dissolved organic matter in the LJW. The same suite of products was observed in photolysates of triclosan in DI water, LJW, and MRW (as determined by HPLC), indicating that the same degradation pathways were operative in both DI and natural water photolyzes.

Although reaction with $^1O_2$ or hydroxyl radical can be made to dominate over direct photolysis under selected laboratory conditions (e.g., high-efficiency sensitizer and screening of UV light), our results indicate that direct photolysis will be dominant in most natural systems. The clearest evidence for this comes from side-by-side photolysis of triclosan in natural wa-

ter samples and DI water, in which no enhancement due to reactive oxygen species was observed in the natural water samples. In pH 8 water bodies with high steady-state $^1O_2$ concentrations ($[^1O_2]_{SS}$, 10^{-12} M [27]), however, the calculated pseudo first-order half-life because of reaction with singlet oxygen alone is 2 h. Under these conditions, triclosan degradation via reaction with $^1O_2$ is expected to be more facile than direct photodegradation ($t_{1/2}$ = 5 h). At the same high $[^1O_2]_{SS}$, direct photolysis is expected to be more competitive with singlet oxygenation as the pH is lowered, because the direct photolysis rate constants are slightly less sensitive to pH than are the $^1O_2$ k_{rxn} values. The competitiveness of the singlet oxygen reaction with direct photolysis is expected to hold only under basic conditions with high $[^1O_2]_{SS}$. Despite its high k_{OH}, degradation of triclosan by hydroxyl radical is expected to be kinetically insignificant because of the extremely low steady-state ·OH concentrations ($<10^{-16}$ M [27]) typically observed in natural waters.

Implications of the present study. The data presented here indicate that triclosan is rapidly degraded ($t_{1/2}$ = 5 h) in surface waters (pH 8) at 45°N latitude under summertime sunlight, which agrees well with field studies that found photochemical degradation to be the major summertime epilimnetic loss process [6,8]. The photochemical reactivity will decrease as the pH is lowered and the less photoreactive phenol form dominates triclosan speciation. The photochemical half-lives will be sensitive to seasonal and latitudinal variations in light intensities, which have been tabulated [29]. Triclosan does not convert to a single photoproduct but, rather, undergoes a number of transformations, including dechlorination [19], ether cleavage, cyclization, and coupling. In the present work, the toxic 2,8-DCDD and 2,4-DCP have been identified as photoreactive products of triclosan photolysis, and their formation kinetics and environmental concentrations are tied to triclosan input and sunlight intensity. We also found evidence that photochemical coupling of triclosan to dissolved organic matter may be a significant process in sunlit natural waters.

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REFERENCES

1. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
2. Lindström A, Buerge IJ, Poiger T, Bergqvist P-A, Müller MD, Buser H-R. 2002. Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. *Environ Sci Technol* 36:2322–2329.
3. Halling-Sorensen B, Nielsen SN, Lanzky PF, Ingerslev F, Lutzhft HCH, Jorgensen SE. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—A review. *Chemosphere* 36:357–393.
4. Daughton CG, Ternes TA. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ Health Perspect Suppl* 107:907–938.
5. Boreen AL, Arnold WA, McNeill K. 2003. Photodegradation of pharmaceuticals in the aquatic environment: A review. *Aquat Sci* 65:320–341.
6. Singer H, Müller S, Tixier C, Pillonel L. 2002. Triclosan: Occurrence and fate of a widely used biocide in the aquatic environment: Field measurements in wastewater treatment plants, surface waters, and lake sediments. *Environ Sci Technol* 36:4998–5004.
7. Tixier C, Singer HP, Oellers S, Müller SR. 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ Sci Technol* 37:1061–1068.
8. Tixier C, Singer HP, Canonica S, Müller SR. 2002. Phototransformation of triclosan in surface waters: A relevant elimination process for this widely used biocide—Laboratory studies, field measurements, and modeling. *Environ Sci Technol* 36:3482–3489.
9. Schweizer HP. 2001. Triclosan: A widely used biocide and its link to antibiotics. *FEMS Microbiol Lett* 202:1–7.
10. Choudhry GG, Webster GRB. 1987. Environmental photochemistry of polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs): A review. *Toxicol Environ Chem* 14:43–61.
11. Freeman PK, Srinivasa R. 1983. Photochemistry of polychlorinated phenoxyphenols: Photochemistry of 3,4,5,6-tetrachloro-2-(pentachlorophenoxy)phenol. *J Agric Food Chem* 31:775–780.
12. Freeman PK, Srinivasa R. 1984. Photochemistry of polychlorinated phenoxyphenols. 3. Solvent effects on the photochemical transformations of 3,4,5,6-tetrachloro-2-(pentachlorophenoxy)phenol. *J Agric Food Chem* 32:1313–1316.
13. Freeman PK, Hatlevig SA. 1994. Photodehalogenation of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin. In Helz GR, Zepp RG, Crosby DG, eds. *Aquatic and Surface Photochemistry*. Lewis, Boca Raton, FL, USA, pp 181–186.
14. Kanetoshi A, Ogawa H, Katsura E, Kaneshima H, Miura T. 1988. Formation of polychlorinated dibenzo-*p*-dioxin from 2,4,4′-trichloro-2′-hydroxydiphenyl ether (Irgasan DP300) and its chlorinated derivatives by exposure to sunlight. *J Chromatogr* 454: 145–55.
15. Kanetoshi A, Ogawa H, Katsura E, Kaneshima H, Miura T. 1992. Study on the environmental hygienic chemistry of chlorinated 2-hydroxydiphenyl ethers: Photolytic conversion to polychlorinated dibenzo-*p*-dioxins. *Kankyo Kagaku* 2:515–22.
16. Latch DE, Packer JL, Arnold WA, McNeill K. 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *J Photochem Photobiol A* 158:63–66.
17. Onodera S, Ogawa M, Suzuki S. 1987. Chemical changes of organic compounds in chlorinated water. XIII. Gas chromatographic-mass spectrometric studies of the reactions of Irgasan DP 300 [5-chloro-2-(2,4-dichlorophenoxy)phenol] with chlorine in dilute aqueous solution. *J Chromatogr* 392:267–75.
18. Sabaliunas D, Webb SF, Hauk A, Jacob M, Eckhoff WS. 2003. Environmental fate of triclosan in the river aire basin, UK. *Water Res* 37:3145–3154.
19. Ferrer I, Mezcua M, Gomez MJ, Thurman EM, Agueera A, Hernando MD, Fernandez-Alba AR. 2004. Liquid chromatography/time-of-flight mass spectrometric analyses for the elucidation of the photodegradation products of triclosan in wastewater samples. *Rapid Commun Mass Spectrom* 18:443–450.
20. Latch DE, Stender BL, Packer JL, Arnold WA, McNeill K. 2003. Photochemical fate of pharmaceuticals in the environment: Cimetidine and ranitidine. *Environ Sci Technol* 37:3342–3350.
21. Packer JL, Werner JJ, Latch DE, McNeill K, Arnold WA. 2003. Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquat Sci* 65: 342–351.
22. Pemberton RM, Hart JP. 1999. Electrochemical behavior of triclosan at a screen-printed carbon electrode and its voltammetric determination in toothpaste and mouth rinse products. *Anal Chim Acta* 390:107–115.
23. Tratnyek PG, Hoigné J. 1991. Oxidation of substituted phenols in the environment: A QSAR analysis of rate constants for reaction with singlet oxygen. *Environ Sci Technol* 25:1596–604.
24. Pförtner K, Boese D. 1970. Photosensitized oxidation of monohydric phenols to quinones. *Helv Chim Acta* 53:1553–66.
25. Wilkinson F, Helman WP, Ross AB. 1995. Rate constants for the decay and reactions of the lowest electronically excited singlet state of molecular oxygen in solution. An expanded and revised compilation. *J Phys Chem Ref Data* 24:663–1021.
26. Buxton GV, Greenstock CL, Helman NP, Ross AB. 1988. Critical review of rate constants for reaction of hydrated electrons and

hydroxyl radicals in aqueous solution. *J Phys Chem Ref Data* 17:513–886.
27. Cooper WJ, Zika RG, Petasne RG, Fischer AM. 1989. Sunlight-induced photochemistry of humic substances in natural waters: Major reactive species. In Suffet IH, MacCarthy P, eds, *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants.* American Chemical Society, Washington, DC, pp 333–362.
28. Boreen AL, Arnold WA, McNeill K. 2004. Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups. *Environ Sci Technol* 38: 3933–3940.
29. Leifer A. 1988. *The Kinetics of Environmental Aquatic Photochemistry: Theory and Practice.* American Chemical Society, Washington, DC.
30. Boehme K, Brauer HD. 1992. Generation of singlet oxygen from hydrogen peroxide disproportionation catalyzed by molybdate ions. *Inorg Chem* 31:3468–3471.
31. Saitz CB, Valderrama JA, Tapia R, Farina F, Paredes MC. 1992. Synthesis of 3,4-dihydro-4-hydroxy-2,2-dimethyl-2H-1-benzopyran-5,8-dione. A potential key intermediate for the preparation of naphtho[2,3-β]pyranoquinones. *Synth Commun* 22:955–960.
32. Chin Y-P, Aiken G, O'Loughlin E. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ Sci Technol* 28:1853–1858.
33. Zepp RG, Cline DM. 1977. Rates of direct photolysis in aquatic environment. *Environ Sci Technol* 11:359–366.
34. Dulin D, Mill T. 1982. Development and evaluation of sunlight actinometers. *Environ Sci Technol* 16:815–820.
35. Haag WR, Hoigné J. 1986. Singlet oxygen in surface waters. 3. Photochemical formation and steady-state concentrations in various types of waters. *Environ Sci Technol* 20:341–348.
36. Burrows HD, Ernestova L, Kemp TJ, Skurlatov YI, Purmal AP, Yermakov AN. 1998. Kinetics and mechanism of photodegradation of chlorophenols. *Prog React Kinet* 23:145–207.
37. Beger RD, Wilkes JG. 2001. Models of polychlorinated dibenzodioxins, dibenzofurans, and biphenyls binding affinity to the aryl hydrocarbon receptor developed using ^{13}C NMR data. *J Chem Inf Comput Sci* 41:1322–1329.
38. Sijm DTHM, Opperhuizen A. 1988. Biotransformation, bioaccumulation and lethality of 2,8-dichlorodibenzo-*p*-dioxin: A proposal to explain the biotic fate and toxicity of PCDDs and PCDFs. *Chemosphere* 17:83–99.
39. McConnell EE, Moore JA, Haseman JK, Harris MW. 1978. The comparative toxicity of chlorinated dibenzo-*p*-dioxins in mice and guinea pigs. *Toxicol Appl Pharmacol* 44:335–356.
40. Okumura T, Nishikawa Y. 1996. Gas chromatography-mass spectrometry determination of triclosans in water, sediment, and fish samples via methylation with diazomethane. *Anal Chim Acta* 325: 175–184.
41. Boule P, Guyon C, Lemaire J. 1984. Photochemistry and the environment. VIII. Photochemical behavior of dichlorophenols in a dilute aqueous solution. *Chemosphere* 13:603–612.
42. Zhang H, Huang C-H. 2003. Oxidative transformation of triclosan and chlorophene by manganese oxides. *Environ Sci Technol* 37: 2421–2430.